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ROOTS AND THE TAXONOMIC DIFFERENCES BETWEEN BOTRYCHIUM ONEIDENSE AND B. DISSECTUM

W. H. WAGNER, JR.[1]

Since first distinguished nearly sixty years ago, the plant here treated as *Botrychium oneidense* (Gilbert) House has been a continuous source of taxonomic disagreement. It has been interpreted as a variety of *B. multifidum* (Gmel.) Rupr. or as a variety of *B. dissectum* Spreng. (Wagner, 1960a). It was originally described as a variety of a third species, *B. ternatum* (Thunb.) Sw. Only House (1921) seems to have recognized *B. oneidense* as a distinct species; but he changed his mind just three years later, and made it a vari-

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ety of *B. obliquum* Muhl. (1924). In general, then, we can find agreement on only one point, viz., that — whatever it is — taxon *oneidense* must be a variety of some other species. No author, at least for any length of time, seems to have thought that *B. oneidense* is truly a distinct species, or that the difficulty of placing it with one or another of the several species of which it has been made a variety might be due to that fact.

After personal observations of over sixty localities where the plants grow together in Ohio, Ontario, and Michigan, as well as fresh, living materials sent by others from southern Indiana and Maryland — a total of over 4,500 specimens of *B. dissectum* and 2,000 of *B. oneidense* — I feel convinced that these are distinct species (Wagner, 1955, 1960a). *Botrychium dissectum* is a highly variable plant, the most common and "normal" form of which is f. *obliquum* (Muhl.) Fern. The type form, *dissectum*, is often so lacerated that botanists have frequently treated it as a variety, or even as a species, distinct from taxon *obliquum*. However, I cannot find any differences between f. *dissectum* and f. *obliquum* other than those of marginal cutting, and there are numerous intermediates. In spite of the rather spectacular contrast in their appearance, I cannot treat even the extremes as more than minor forms of the same species. On the contrary, *B. multifidum*, *B. oneidense*, and *B. ternatum* each have a series of differences from one another and from *B. dissectum* that justify their interpretation as distinct species. This conclusion is substantially bolstered by the fact that all four of these species co-exist side-by-side in the same habitats in any combination, over a tremendous area of the northeastern United States (Wagner, 1960a, 1960b). The most significant point is perhaps that, in spite of overlap in practically every character that differentiates these plants, there are correlated series of central tendencies for each taxon that in totality are clearly different. These ensembles of average differences maintain themselves, whereever these plants grow together, with monotonous regularity. I do not, in fact, believe that all of the differences between them have yet been found. The present report will

PLATE 1259. Habitat forms of *Botrychium oneidense:* Top two rows, deep shade, Saginaw Co., woods along M-83, Sect. 22, R.6E, T.10N, Sept. 20, 1959, 9110. Bottom two rows, edges of cleared old fields, St. Clair Co., along Belle River, Sect. 28, T.5N, R.15E, May 12, 1957, 8393.

PLATE 1260. Habitat forms of *Botrychium dissectum:* Top two rows (9109) and bottom two rows (8394) from same localities as Plate 1259.

describe a newly uncovered contrast between two of the species that was quite unexpected and apparently completely overlooked, not only by previous workers but by the present author as well. It involves the root differences between *Botrychium oneidense* and *B. dissectum*.

In making studies of critical characters among these species, *the populations must be compared in the same habitats* (e.g., a shaded swamp; a low, wet, brushy field; or a second-growth wood). Differing localities and differing habitats produce strong modifications as shown in Plates 1259 and 1260, which will be discussed below. To ensure as precise a comparison as possible the rule followed in this study was to collect *only paired plants of the two species.* To obtain each pair, a spot was sought where a plant of *B. oneidense* grew close to a comparable (i.e., of approximately equal size) plant of *B. dissectum*. The arbitrary limit of permissible distance between them was five feet. Then the plant of *B oneidense* was dug up, along with the neighboring one of *B. dissectum*. Thus the average distance between the members of the pairs was between two and three feet. (The same rule was followed to compare other species, to be mentioned below.) The measurements of the diameters of the two largest roots of each living specimen were made 1 cm. from the stem to the nearest 0.1 mm., using calipers.

RESULTS. The habitat variation of roots in *B. oneidense* is probably fairly well represented by the data of this study, but the same cannot necessarily be said for *B. dissectum*. The reason for this is that the morphological differences found between various localities of *B. oneidense* are considerably less pronounced than those in the other species of evergreen grapeferns (*B. dissectum*, *B. multifidum*, and *B. ternatum*). *Botrychium oneidense* seems to be the least variable of the species. The reason for its lesser environmental variability seems very likely to be due to its narrower definition of habitat: it is confined almost exclusively to low, wet, acid secondary woods and swamps. The most extreme leaf forms that have been found in this species are illustrated in Plate 1259. The lower two rows are from the most exposed habitat we have yet discovered — exposed

mossy areas along the edge of a dry field bordering a woods. The upper two rows are from a very deeply shaded habitat along the edge of a swamp. Corresponding specimens of *B. dissectum* from the same habitats are shown in Pl. 1260. Much more extreme specimens are known of the latter species. The five habitats we selected in which to compare the roots of these two species are what might be called approximately average for *B. oneidense* — neither the most exposed nor the most deeply shaded.

Differences between the means in root diameter of the two taxa in the different localities turned out to vary from 0.5 to 1.0 mm., as shown in Table 1. The roots of *B. dissectum* were always larger. This difference is readily evident to the naked eye when examining the root systems of a series of freshly collected specimens that have been washed. The greater thickness of the roots of *B. dissectum* is accentuated by the fact that they tend to be dark gray-brown in the region 1-5 cm. from the stem especially, but those of *B. oneidense* are paler, more delicate, and dominantly ivory-gray. Also the roots of the latter tend to have much less

TABLE 1. Root diameters of mature plants, the two largest roots of each plant measured 1 cm. from the stem. All (except *) paired specimens in same habitat.

	B. dissectum	*B. oneidense*
1.	9218a (5 plants)	9219a (5 plants)
	2.87	2.10
2.*	9218b (9)	9219b (10)
	3.08	2.15
3.	9263 (14)	9264 (14)
	2.90	2.20
4.	9265 (19)	9266 (19)
	2.92	2.20
5.	9289 (28)	9290 (28)
	3.05	2.53
6.	9307 (18)	9309 (18)
	3.06	2.48

developed circular ridges on the roots. This is shown in the samples in Plate 1261.

An anatomical examination was made of the histological nature of these differences. Specimens fixed in formalin-

acetic-alcohol solution sectioned nicely on the freezing microtome, and were examined under the compound microscope. The roots of both species are very fleshy, composed of a large cortex of starch-filled parenchyma cells and traversed by a narrow stele, the latter only 10-15 per cent the diameter of the whole root. The root steles of both are most commonly triarch, although the tetrarch condition is frequent near the rhizome, and small roots may be diarch. The primary variation of the roots at the anatomical level seems to involve the relative development of the cortex and the modification of the superficial layers. A thin-walled corky tissue

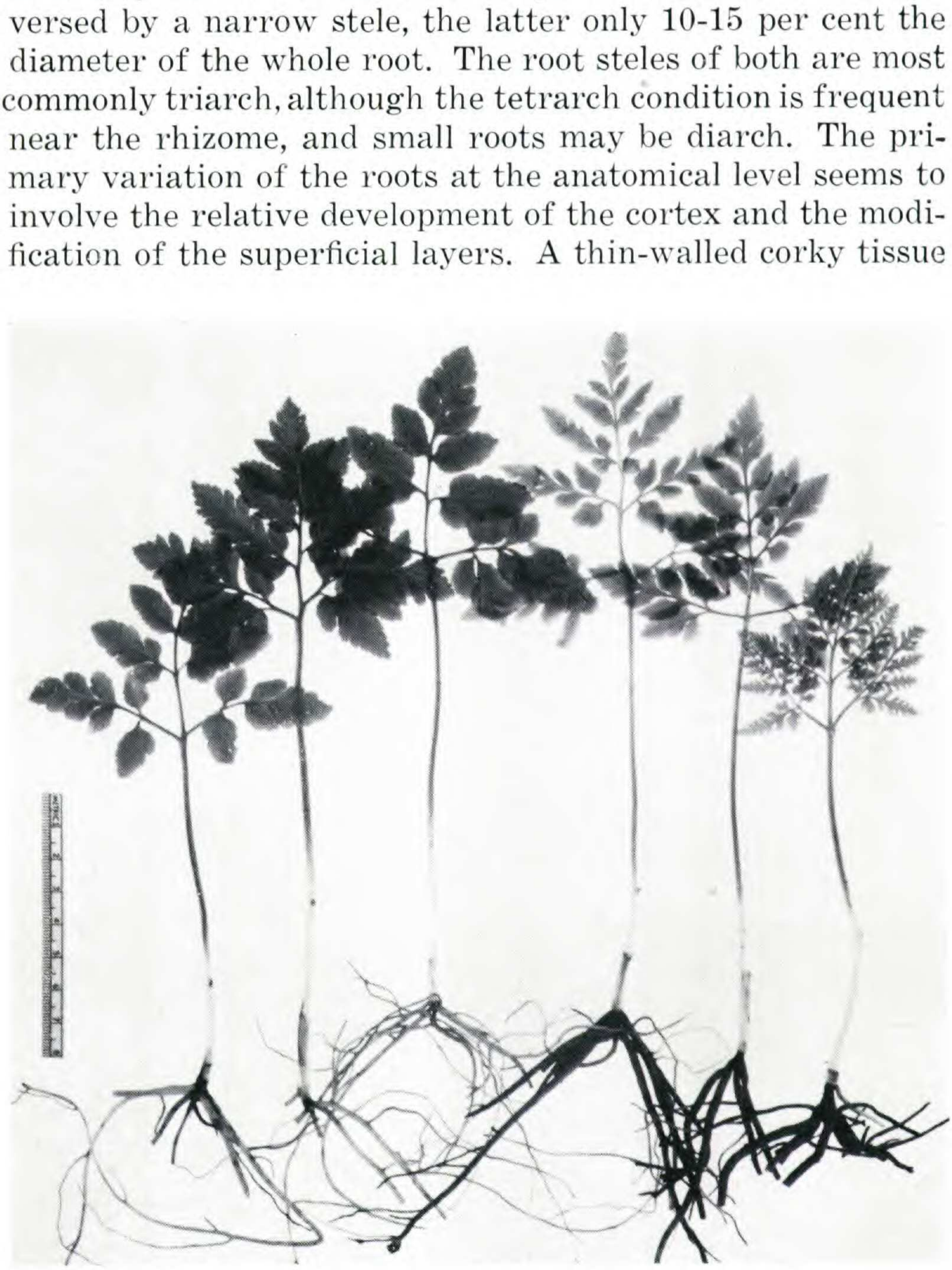

PLATE 1261. Root systems of Botrychium: Three plants on left, 9264, *B. oneidense;* three on right, 9263, *B. dissectum.* (Photographed on frosted glass plate, with light from above and below).

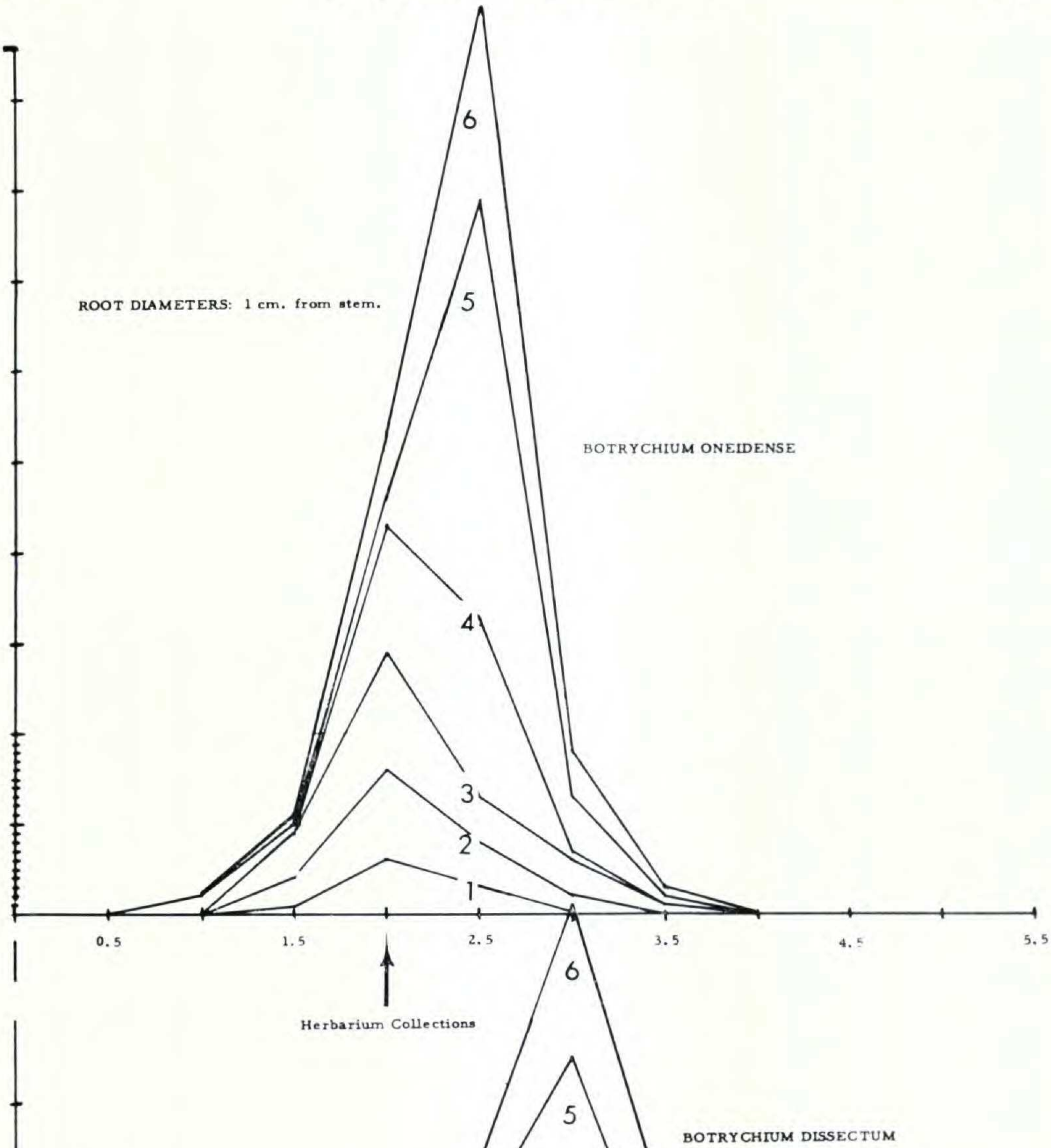

FIGURE 1. Root diameters of Botrychium. Curve numbers correspond to Table 1. Vertical scale represents number of individuals.

forms in a varying number of layers from a cambium in the outer cortex, and this tissue, comprising dead, brown-walled cells, is responsible for the darker color of the roots of *Botrychium dissectum*. The same development is found in *B. oneidense*, but its extent is less, and thus the roots appear much paler on the average and have smoother surfaces. The development of the root periderm is associated with the formation of circular ridges, these more strongly developed in *B. dissectum* but only weakly developed in all but the most robust roots of *B. oneidense*.

The differences between *B. dissectum* and the other species of evergreen grapeferns, *B. multifidum* and *B. ternatum*, proved to be negligible or inconsistent. In drier field habitats, *B. multifidum* exceeded the associated *B. dissectum* in root diameter, but in very damp habitats (two localities, one at the edge of a bog, the other in low swampy woods) it tended to have narrower roots than *B. dissectum*. *Botrychium ternatum* in three localities had roots the same thickness or somewhat more slender than those of *B. dissectum* growing with it. In general, the roots of *B. multifidum* and *B. ternatum* are more like those of *B. dissectum*; only *B. oneidense* showed a constant average difference, so far as our studies have been able to show.

In order to test whether the difference in root diameter between *B. dissectum* and *B. oneidense* would also be shown in random herbarium collections, 18 specimens each of the two species were measured from the United States National Herbarium. The results showed an average of 2.6 mm. in diameter in the former, and 2.0 mm. in the latter, as measured 1 cm. from the stem. A remeasurement of dried materials that have been compared in the living state first shows that there is almost exactly 20 per cent reduction in thickness. The original differences between species will thus remain in the dry state but on a somewhat smaller scale. According to this contraction of dried material, the National Herbarium samples would have averaged in the living state 3.1 mm. in diameter for *B. dissectum* and 2.4 for *B. oneidense*. These values compare favorably with those in Table 1.

DISCUSSION. Although this report is based upon a sample of 187 plants from only 5 habitats, the methods used seem to be sufficiently reliable to say that the average difference we found is a valid one. The fact that a random collection of herbarium sheets gave essentially the same results is an indication that the root differences between *B. dissectum* and *B. oneidense* are probably characteristic of the two species over their range.

One of the reasons that special care has been taken to assure that the differences between the roots are not environmentally induced ones is that there is some reason to believe that several of the previously accepted "differences"

TABLE 2. Summary of average differences of *B. oneidense* and *B. dissectum*.

	B. oneidense	*B. dissectum*
Habitat:	Mainly uniform: low, wet, acid secondary woods and swamps; local and rare in most of its area.	Diverse: open fields, brushy meadows, dry woods, wet woods, swamps; ubiquitous and common in much of its area.
Range:	Narrow: New Brunswick and Wisc. S. to Indiana and Maryland; and to North Carolina and Tennessee in the mountains.	Wide: Nova Scotia and Minnesota S. to Mexico and Jamaica.
Leaf Blade:	Segments few, large, rounded, with nearly entire to shallowly denticulate margins. Mature surfaces duller, smoother (less "veiny").	Segments numerous, smaller, pointed, with variable margins denticulate to very deeply and coarsely lacerate. Mature surfaces shiny, "veiny."
Pigmentation:	Unfolding leaves in spring lime green; late summer mature blade axes with av. 3-15 per cent pigmentation below; midwinter laminae mostly green where well exposed.	Unfolding leaves in spring reddish; late summer mature blade axes with av. 10-40 per cent pigment beneath; midwinter laminae bronze where well exposed.
Periodicity:	Vernation beginning in May, the new leaf averaging twice as tall during vernation stages; maturation of sporangia in last half of September.	Vernation beginning in early June, the new leaf averaging one-half as tall during vernation stages; maturation of sporangia in October.
Roots:	Root diameter 1 cm. from stem av. 2.5 mm. alive, 2.0 dried; circular ridges sparse in proximal 1-5 cm.; color dominantly ivory-gray to tan, only occasional large roots completely dark in basal 1-5 cm.	Root diameter 1 cm. from stem av. 3.0 mm. alive, 2.5 mm. dried; circular ridges well developed in proximal 1-5 cm.; color dominantly dark gray-brown in proximal 1-5 cm.

between these species are actually based upon habitat modifications. For example, we have found no evidence that *B. oneidense* has a distinctively "thin texture of the blade" when growing with *B. dissectum* (Wagner, 1960b, table 4, p. 318). Likewise, we have found no evidence of a "marked tendency toward lower fertility": indeed, when growing sympatrically in the habitats, the two species are very much alike (Wagner, 1961). We examined the spores to find differences but they too are similar.

However, there are a number of real average differences between these plants. It is, of course, possible that one or a few important genes could somehow control all of the differences, but this seems unlikely because the characters involved are so diverse. The differences lie in six categories (listed in Table 2). It is probable that many of the details of contrasts within several of the categories are inter-related and are aspects of the same thing: for example, the three contrasts under "roots" are probably all correlated. Those under periodicity, under pigmentation, and under leaf structure, may be also — but this is questionable. For example, the size and the shape of segments must surely be under different genetic control, judging from these characters in the other species. Therefore, the best statement of the differences between these two species, *B. oneidense* and *B. dissectum*, that we can make (including here the new root characters, as well as those studied previously) is as follows: they are sympatric species in northeastern North America, the range of one *(B. dissectum)* completely overlapping the other *(B. oneidense)*, and they differ in the central tendencies of differences in six known categories: (1) Habitat; (2) Range; (3) Leaf blade structure; (4) Pigmentation; (5) Periodicity; and (6) Root size and development.

VOUCHER SPECIMENS: (All Michigan field collections). Wayne Co., junct. Flat Rock Rd. and Expressway, R.9E, T.3S, Sect. 19, May 20, 1960, 9218a (Dissectum), 9219a (Oneidense), 9218b and 9219b unpaired in same habitat. Monroe Co., woods W. side of Secor Rd., 0.4 mi. s. of Todd Rd., R.7E, T.8S, Sect. 30, August 4, 1960, 9263 (D), 9264 (O). Monroe Co., S.W. corner of Nolan and Exeter Rd.,

R.8E, T.5S, Sect. 24, August 4, 1960, 9265 (D), 9266 (O). Saginaw Co., Fordney R., N. of Brady Rd., Sect. 11, R.2E, T.9N, Aug. 13, 1960, 9289 (D), 9290 (O). St. Clair Co.: N. side of Rt. 21, 0.4 mi. W. of Beach Rd., Sect. 6, R.17E, T.6N, August 23, 1960, 9307 (D), 9309 (O). — UNIVERSITY OF MICHIGAN, ANN ARBOR.

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TWO NEW ORCHID RECORDS FOR ONTARIO. — While orchid hunting last year, I met Mrs. J. C. Higgins of Komoka, Ont., who told me that she had *Liparis lilifolia* growing in her woodlot. I was skeptical of her identification, since this species had not been reported previously in Ontario, and since the province is not included in the range given for the species in Gray's Manual. I was invited to visit the site this year, and on June 18th, accompanied by several friends, I went to Komoka and was delighted to see *Liparis lilifolia* (L.) Richard for the first time. It was growing in a predominantly beech-maple woods on the southwest facing slope of the Oxbow Creek, among herbaceous species usually found in this type of woods. Mrs. Higgins told us that this year she had counted 83 plants and she first noticed the orchid in this vicinity about 10 to 15 years ago. Photographs were taken, and Prof. Montgomery took one specimen for the herbarium of the Ontario Agricultural College, Guelph.